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Electrophoretic Comparison of European *Dendroctonus micans* and Ten North American *Dendroctonus* Species (Coleoptera: Scolytidae)

MOLLY W. STOCK, JEAN-CLAUDE GRÉGOIRE and MALCOLM M. FURNISS

Department of Forest Resources, University of Idaho, Moscow, Idaho 83843; Laboratoire de Biologie Animale et Cellulaire, Université Libre de Bruxelles, 1050 Brussels, Belgium; Department of Plant, Soil, and Entomological Sciences, University of Idaho, Moscow, Idaho 83843

Abstract.—Electrophoretic techniques were used to estimate genetic relationships among the great European spruce bark beetle, *Dendroctonus micans* (Kugelann), and 10 North American *Dendroctonus* species. Average heterozygosity for *D. micans* was .053; the North American species ranged from .114 to .226. Cluster analysis suggests that *D. micans* is more closely related to *D. terebrans* and *D. valens* than to other species in the genus.

The great European spruce bark beetle, *Dendroctonus micans* (Kugelann), occurs in spruce forests of Eurasia from the United Kingdom to Siberia. It is believed to have originated in Siberia from a North American spruce-feeding ancestor (Wood 1963) and subsequently spread across Europe. It was discovered in England in 1982 (Bevan and King 1984, Evans et al. 1984).

Relationships among adult *Dendroctonus* species have been studied by Hopkins (1909), Wood (1963, 1982), and Lanier (1981). Immatures were compared by Thomas (1965). Except for its greater size, *D. micans* appears virtually identical to the North American *D. punctatus* LeConte, and may be conspecific (Bright 1976). *D. micans* (Bevan and King 1984), and probably *D. punctatus,* are similar to *D. valens* LeConte, *D. terebrans* (Olivier), and *D. rhizophagus* Thomas and Bright in that adults construct egg galleries in the lower bole and large roots and the larvae mine communally rather than make discrete individual tunnels. However, morphologically (Hopkins, 1909, Wood 1982) and cytogenetically (Lanier 1981) *D. micans* and *D. punctatus* have been allied with *D. rufipennis* Kirby and *D. murrayanae* LeConte. Some aspects of the biology and behavior of *D. micans* are unusual. For example, successful attacks by solitary females are common and inbreeding is the rule. Vouland et al. (1984) report that usually more than 90% of the females are fertilized before they emerge.

Electrophoretic studies of 10 representative North American *Dendroctonus* species revealed species clusters very similar to those developed using cytogenetic and anatomic evidence (Bentz and Stock 1986). *D. micans* and *D. punctatus,* however, were not available for inclusion in those studies. Preliminary electrophoretic comparisons of *D. micans* from France and Great Britain have revealed consistent differences within this species (Evans et al. 1984). More recently,

acquisition of live *D. micans* from Belgium permitted electrophoretic comparison with the 10 *Dendroctonus* species studied earlier. Results of this comparison are reported here.

Methods

Larvae were grown in the laboratory in Belgium from females introduced into fresh *Picea excelsa* (*P. abies*) logs. Fifth instar larvae were shipped from Belgium and received at the University of Idaho, on May 25, 1985, in a layer of fresh phloem taped tightly between two 15 × 15 cm pieces of plate glass. Pupae were noted beginning June 4 and all had transformed to adults by June 25. On June 27 and July 5, two lots of 50 females each were frozen at −30° C.

Electrophoretic analysis of these beetles followed methods described by Higby and Stock (1982) and Bentz and Stock (1986) for other *Dendroctonus* species. Genetic diversity was estimated and compared among groups using percent polymorphism and Nei's (1975) average heterozygosity. A locus was considered polymorphic when the frequency of the common allele was less than or equal to .99. Relationships between *D. micans* and other *Dendroctonus* species were assessed, using BIOSYS-1 (Swofford and Selander 1981), by hierarchical cluster analysis of Nei's (1978) genetic distance values.

Results and Discussion

Data from 15 gene loci were obtained (Table 1). Of these, four (AAT, IDH-1, ME, and PEP-gl) were polymorphic, and 11 (CK, EST-1, EST-2, EST-4, IDH-2, MDH-1, MDH-2, MPI, PEP-la, PGI, and SOD) were monomorphic. These beetles were thus much less genetically diverse (27% polymorphism) than any other *Dendroctonus* species that have been studied electrophoretically: polymorphism over the same 15 loci in 10 other species ranged from 40% to 67%. Average heterozygosity for the species was .053, compared to a range of .114 to .226 in the other 10 *Dendroctonus* species at these 15 loci. Cluster analysis suggests that *D. micans* is more closely related to *D. terebrans* and *D. valens* than to other species in the genus (Figure 1).

Specimens of *D. micans* used in this study were from broods of only two females but may still represent the species' diversity. Because the species is isolated from others of the genus and inhabits a unique niche in European forests, with little or no

Table 1. Allele frequencies at 15 gene loci[1] in *Dendroctonus micans* and *D. ponderosae*. Data for *D. ponderosae* are taken from Bentz and Stock (1986). Percent polymorphism (P) and average heterozygosity (H) are also given.

Enzyme	*Locus*	*D. micans*	*D. ponderosae*
AAT	A	.606	—
	B	.387	.662
	C	.007	.338
	(N)	(91)	(334)
CK	B	1.0	.989
	C	—	.011
	(N)	(60)	(323)

Table 1. continued

Enzyme	*Locus*	*D. micans*	*D. ponderosae*
EST-1	A	1.0	1.0
	(N)	(64)	(421)
EST-2	A	—	.019
	B	—	.277
	C	—	.278
	D	—	.137
	E	—	.234
	F	—	.055
	G	.991	—
	H	.009	—
	(N)	(73)	(399)
EST-4	A	—	.993
	B	—	.007
	C	1.0	—
	(N)	(54)	(291)
IDH-1	B	.995	—
	C	.045	1.0
	(N)	(44)	(361)
IDH-2	A	—	1.0
	B	1.0	—
	(N)	(64)	(297)
MDH-1	B	1.0	1.0
	(N)	(58)	(377)
MDH-2	A	—	1.0
	B	—	—
	C	1.0	—
	(N)	(58)	(348)
ME	A	.905	1.0
	B	.095	—
	(N)	(67)	(478)
MPI	B	1.0	1.0
	(N)	(56)	(300)
PEP-gl	A	.989	—
	B	.011	.932
	C	—	.068
	(N)	(44)	(420)
PEP-la	A	1.0	.017
	B	—	.832
	C	—	.151
	(N)	(58)	(235)
PGI	C	—	.009
	D	.009	.978
	E	.991	.014
	(N)	(74)	(404)
SOD	B	1.0	1.0
	(N)	(53)	(421)
P		27	40
H		.053	.114

[1]AAT = aspartate aminotransferase, CK = creatine kinase, EST = esterase (three loci), IDH = isocitrate dehydrogenase (two loci), MDH = malate dehydrogenase (two loci), ME = malic enzyme, MPI = mannose phosphate isomerase, PEP-gl = peptidase glycyl-leucine, PEP-la = peptidase leucyl-alanine, PGI = phosphoglucose isomerase, SOD = superoxide dismutase (sometimes called TO or tetrazolium oxidase in other reports).

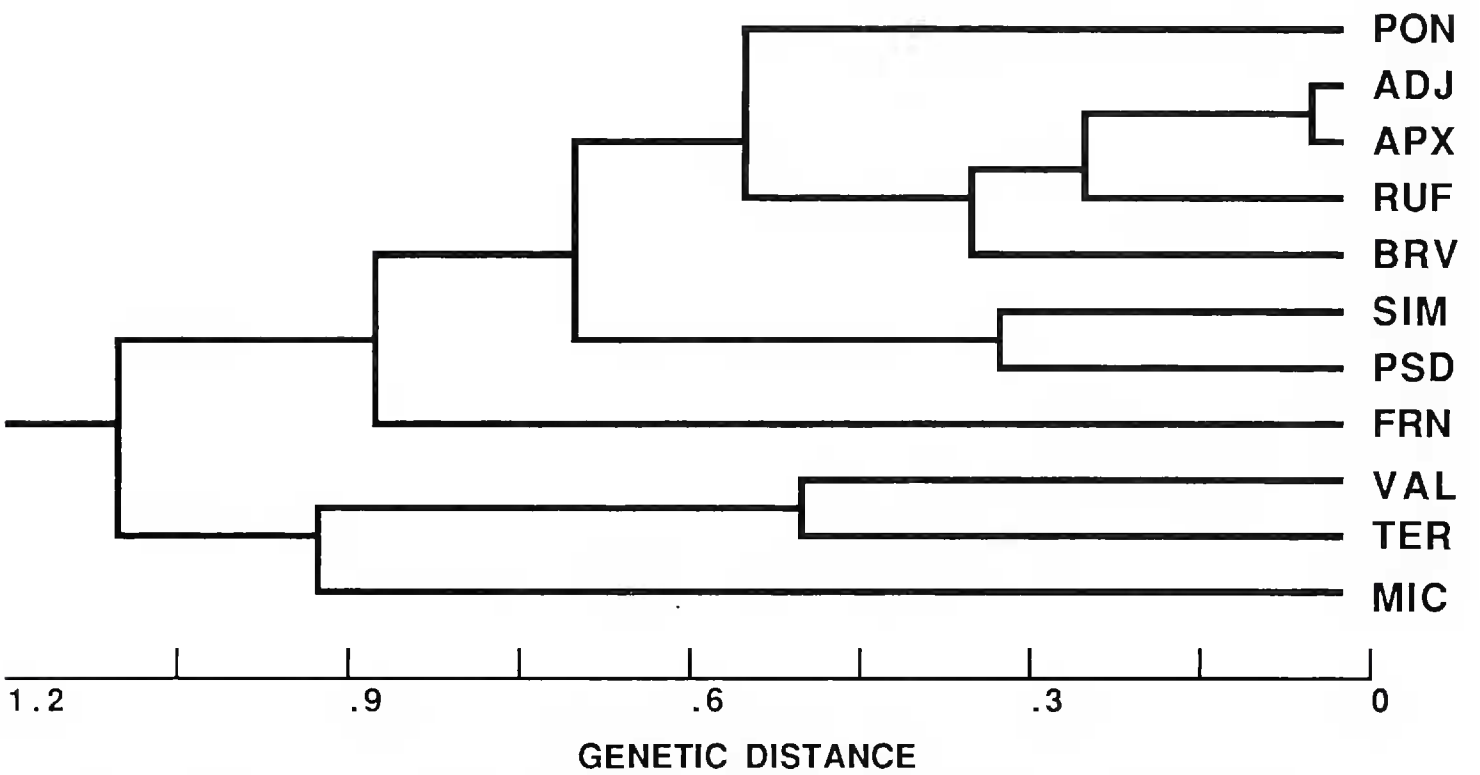

Figure 1. Phenogram derived from electrophoretic data illustrating relationships among 11 *Dendroctonus* species. (PON = *ponderosae,* ADJ = *adjunctus,* APX = *approximatus,* RUF = *rufipennis,* BRV = *brevicomis,* SIM = *simplex,* PSD = *pseudotsugae,* FRN = *frontalis,* VAL = *valens,* TER = *terebrans,* MIC = *micans*)

competition from other insect species, and because the species is highly inbred, it is possible that the low level of genetic diversity observed here may be typical.

Based on the hosts and earlier anatomic and cytogenetic studies, we expected that our electrophoretic data on *D. micans* would correspond most closely to *D. rufipennis*. We observed, however, a closer relationship to the pine-infesting *D. terebrans* and *D. valens* with which *D. micans* shares gregarious larval feeding in the phloem of living hosts.

In order to further clarify *D. micans'* genetic relationship within the genus, we hope to obtain additional broods of *D. micans,* as well as samples of *D. punctatus* and *D. murrayanae* for electrophoretic comparison. Such work has been hindered to date by the relative scarcity of *D. punctatus* and the difficulty of obtaining live specimens of *D. micans* from distant locations.

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